[00128] **CLAIMS**

We claim:

1. A method of cDNA sequencing comprising:

(1) constructing at least one cDNA original library using ribonucleic acid isolated from cells of an organism in which the length of inserted fragments is 0.5-3.0 kb;

(2) homogenizing the cDNA original library according to a graded C_0t value, wherein C_0 is concentration of total DNA (in mol/L) based on the number of nucleic acids; and t is the renaturing time (in seconds);

(3) selecting and sequencing 5-500 clones from the homogenized cDNA library;

(4) synthesizing one or more probes corresponding to clones sequenced in **(3)**, and hybridizing and subtracting the homogenized cDNA library with said probes; and

(5) repeating **(3)** and **(4)** 1-5,000 times.

2. The method of claim 1, wherein the ribonucleic acid is isolated from cells selected from the group consisting of cells at a selected stage of growth, development, or circadian oscillation, cells that display pathological features, and cells that comprise a particular tissue.

3. The method of claim 2, wherein more than one cDNA original library is constructed.

4. The method of claim 3 further comprising,

homogenizing said cDNA original libraries respectively to obtain homogenized libraries of different tissues; and

hybridizing and subtracting among said homogenized cDNA libraries of different tissues.

5. The method of claim 3, wherein the cells used to prepare one cDNA original library differ from the cells used to prepare another cDNA original library(ies) in stage of growth, development, or circadian oscillation, pathological features, and/or tissue of origin.

6. The method of claim 1, wherein (**4**) further comprises:

synthesizing probes based on known cDNA sequences, or synthesizing probes based on other sequenced clones; and hybridizing and subtracting the homogenized cDNA libraries of the preceding step with said probes.

7. The method of claim 1, wherein (**3**) further comprises:

determining whether sequenced fragments are new cDNA clones;

8. The method of claim 7, wherein (**3**) further comprises:

analyzing the integrity of 5' end of new cDNA clones;

9. The method of claim 8, wherein (**3**) further comprises:

sequencing clones that have an intact 5' end until obtaining the full-length sequences.

10. The method of claim 1, wherein there are from 3 to 8 grades of C_0t.

11. The method of claim 10, wherein C_0t is divided into 3 grades: $0 < C_0t < 1$, $C_0t = 1\text{-}50$, and $C_0t > 50$.

12. The method of claim 1, wherein constructing cDNA original libraries comprises:

extracting mRNA, amplifying mRNA to obtain the corresponding cDNA by a technique selected from the group consisting of : a mixed reverse transcriptase technique, a SMART PCR technique, a nucleotide capping technique and combinations thereof;

separating and collecting cDNA fragments of 0.5-3.0kb;

cloning the separated cDNA fragments into suitable vectors;

separating the vectors comprising the inserted fragments of 0.5-3.0kb; and

transforming into suitable bacteria.

13. The method of claim 1, wherein constructing cDNA original libraries comprises:

extracting mRNA, amplifying the mRNA to obtain the corresponding cDNA by a technique selected from the group consisting of : a mixed reverse transcriptase technique, a SMART PCR technique, a nucleotide capping technique and combinations thereof;

separating and collecting cDNA fragments of 0.5-3.0kb;

cloning the separated cDNA fragments into suitable vectors;

separating the vectors comprising the inserted fragments of 0.5-3.0kb;

transforming separated vectors into suitable bacteria; and

extracting DNA from the cDNA libraries, passing the DNA through a poly$(T)_{10-25}$ affinity chromatography column, collecting the cDNA bound with poly$(T)_{10-25}$ and transferring said cDNA into suitable bacteria.

14. The method of claim 10 further comprising separation and collection of cDNA fragments of 0.5-3.0 kb by electrophoresis and gel excision or by gel chromatography

purification; and separation of the vectors comprising the inserted fragments of 0.5-3.0 kb by reversed phase HPLC.

15. The method of claims 11, further comprising separation and collection of cDNA fragments of 0.5-3.0 kb by electrophoresis and gel excision or by gel chromatography purification; and separation of the vectors comprising the inserted fragments of 0.5-3.0 kb by reversed phase HPLC.